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ECTOMYCORRHIZAL MANTLES AS INDICATORS OF HYDROLOGY FOR JURISDICTIONAL WETLAND DETERMINATIONS

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Abstract: Ectomycorrhizae are symbiotic relationships between soil fungi and higher plants. Evidence of the symbiosis is the presence of a 'mantle,' a hyphal layer that covers root tips, and a change in root morphology. The potential use of ectomycorrhizal mantles as hydrology indicators for wetland determinations was evaluated on the Delmarva Coastal Plain (Delaware and eastern shores of Maryland and Virginia, USA) over three seasons. In theory, the distribution of mantles with soil depth should vary from uplands to wetlands in most years, as mantle development is considered to be impeded by anaerobic conditions. At four forested locations, plots were set up in seasonally-saturated wetlands and adjacent uplands and drained wetlands (twelve sub-sites). Plots were evaluated according to the Corps of Engineers Wetlands Delineation Manual for soils, plant community, and hydrology to identify a jurisdictional classification. Hydrology was further addressed using automated monitoring wells (twice daily readings), and anaerobic conditions were confirmed via platinum electrodes. Plant roots (*Pinus taeda* was targeted) were sampled via spade slices in March and August each year and separated by depth: O horizon, 0–5 cm, 5–10 cm, 10–15 cm, and 15–20 cm. Roots were evaluated for the presence of mantles. A threshold depth of 5 cm was identified. From a total of 892 roots with mantles in uplands (including effectively-drained wetland sub-sites), 253 (28%) were found below the threshold depth. For wetlands (including one ineffectively-drained wetland), seven of 331 roots with mantles (2%) were found below the threshold depth. Temporal and spatial variability in mantle data was common; however, mantles consistently occurred at greater depths where seasonally high water tables were lower. We concluded that mantle depth has potential as a hydrology indicator.

Key Words: ectomycorrhizae, mantles, seasonally-saturated wetlands, hydrology indicators, drained hydric soils, wetland delineation

Table 1. Soil classification and hydric soil field indicators.

Sub-sites	Soil Series	Soil Subgroup	Hydric Soil Field Indicator
Redden			
Wetland	Berryland	Typic Alaquods	S7, dark surface
Drained	Mullica	Typic Humaquepts	S7, dark surface
Upland	Atsion	Aeric Epiaquepts	None
Wilder			
Wetland	Fallsington	Typic Endoaquults	F3, depleted matrix
Drained	Fallsington	Typic Endoaquults	F3, depleted matrix
Upland	Woodstown	Aquic Hapludults	None
Ches. Farms A and B			
Wetland	Othello	Typic Endoaquults	F3, depleted matrix
Drained	Othello	Typic Endoaquults	F3, depleted matrix
Upland	Mattapex	Aquic Hapludults	None

INTRODUCTION

A challenging situation in wetland delineation arises when anthropogenic drainage changes the hydrology of a site so that soil morphology may not reflect present hydrologic conditions. This is of particular concern in seasonally-saturated wetlands where wetland hydrology is not evident during much of the growing season. As hydrology is variable with respect to the season and rainfall patterns, it is not the most consistent indicator of wetland status. Plant communities, because of their seasonal nature, are also not consistent indicators of wetland status, especially in areas where the woody strata are dominated by species with a frequency of occurrence in wetlands similar to that in uplands (i.e., facultative (FAC) species) (Reed 1997). Soil flora represent an underutilized source of information concerning wetland status. One group of soil organisms that may have potential as indicators of hydrology are the mycorrhizal fungi.

Mycorrhizal fungi are ubiquitous soil inhabitants that form symbiotic relationships (mycorrhizae) with roots of terrestrial plants from every phylum. In trees of temperate forests, most mycorrhizae are ectotrophic (i.e., a hyphal layer (mantle) covers the root tips and hyphae penetrate between root cells, forming a net-like structure referred to as a 'Hartig net'). The presence of the mantle restricts root elongation but promotes dichotomous branching (Lakhanpal 2000). Therefore, infected roots become stunted, club-shaped, and bifurcate. The mantle tends to be a distinct color that contrasts with that of the root surface. Common mantle colors are white, tan, copper, black, and purple. These morphological features make the mantles easy to identify with the naked eye. These ectomycorrhizae (ECM) are considered critical to the survival and growth of forest trees. The ectomycorrhizal fungi primarily represent the phyla Basidiomycota and Ascomycota (Lakhanpal 2000) and form the symbiosis with tree species from the following families: Pinaceae, Fagaceae, Betulaceae, Myrtaceae, Aceraceae, Cupressaceae, Leguminosae, Rosaceae, Salicaceae, and Ulmaceae (Trappe 1962).

Development of ECM is generally impeded by saturated soil conditions (Slankis 1973).This is due to a number of possible factors. First, ectomycorrhizal fungi are aerobic, and their development is, therefore, inhibited by anaerobiosis (Marks and Foster 1973). Low oxygen tensions render roots less susceptible to infection (Russell 1977). Elevated carbon dioxide levels have been shown to increase the lag phase of growth of ectomycorrhizal fungi (Straatsma et al. 1986). Second, ectomycorrhizal colonization is promoted by low soluble phosphorus levels (Bentivengea and Hetrick 1992). Reducing conditions associated with soil saturation elevate soluble phosphorus levels (Ponnamperuma 1972, D'Angelo and Reddy 1994).

Relatively little research has addressed ectomycorrhizae development in wetlands. In general, the literature reports reduced or complete absence of ectomycorrhizae in permanently inundated anoxic soils and a reduction in ectomycorrhizae in soils with water tables close to the surface (Mejstrik 1976, Kahn and Belik 1995). Mycorrhiza formation under saturated conditions has been reported, but usually arbuscular mycorrhizal fungi (AM, which do not produce mantles) are involved (Lodge 1989). There are, however, certain ECM fungi that infect *Alnus* and *Salix* and appear to be adapted to continuously wet soil conditions (Trappe 1977, Marshall and Pattullo 1981). It is assumed that ECM development under those conditions is facilitated by oxygen diffusion from the roots. Also, Lodge and Wentworth (1990) reported that AM fungi

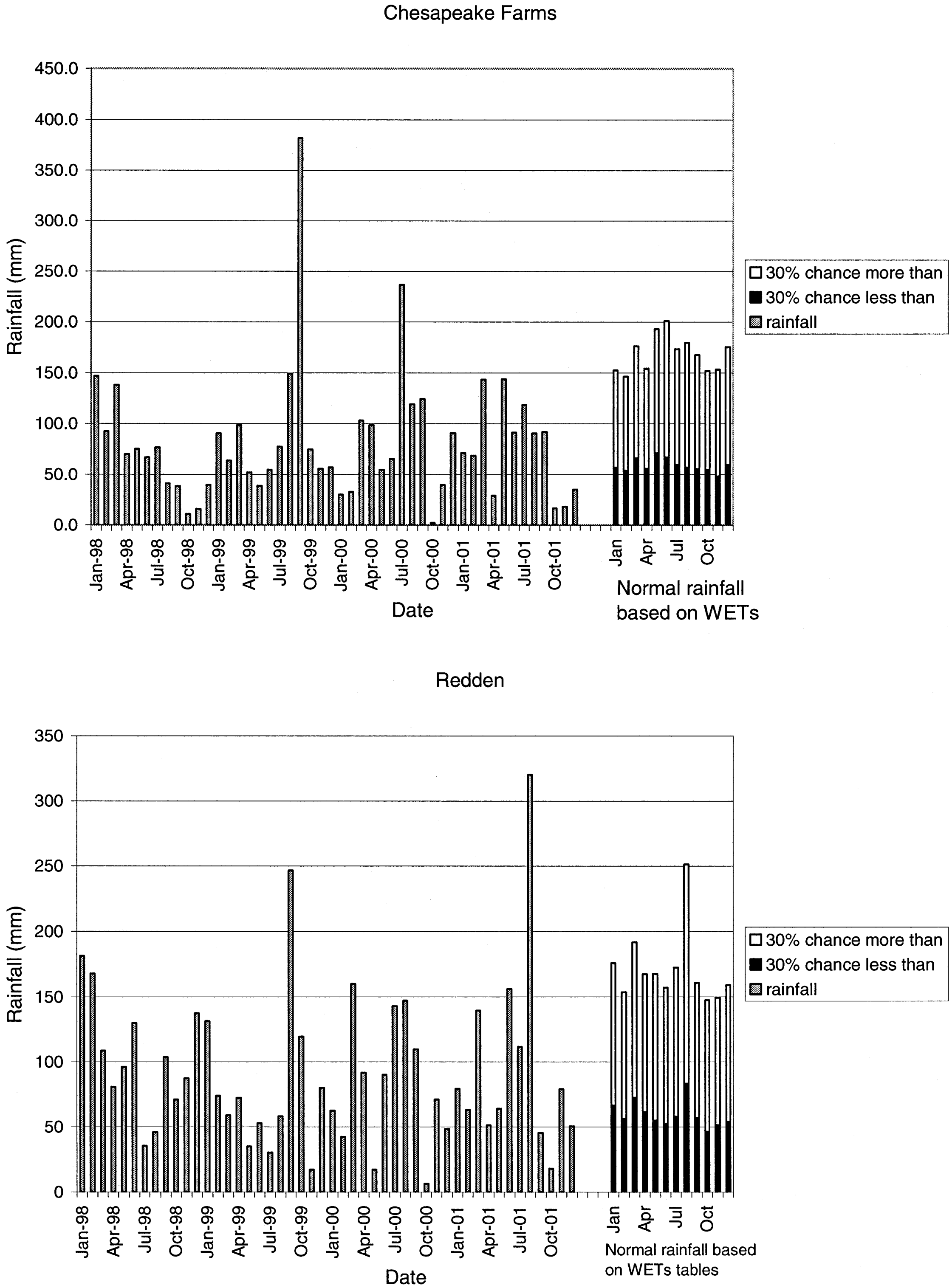

Figure 1. Monthly and long-term precipitation.

displaced ECM fungi on tree roots as the soil became saturated.

Our premise is that the vertical distribution of ECM mantles in the soil should reflect the seasonally-high water table. Therefore, ECM mantles are a potential hydrology indicator useful in identifying whether a site has been drained. This would be useful in areas with stratified sediments where ditching may be lowering the apparent water table but wetland hydrology is maintained through episaturation. As such, ECM man-

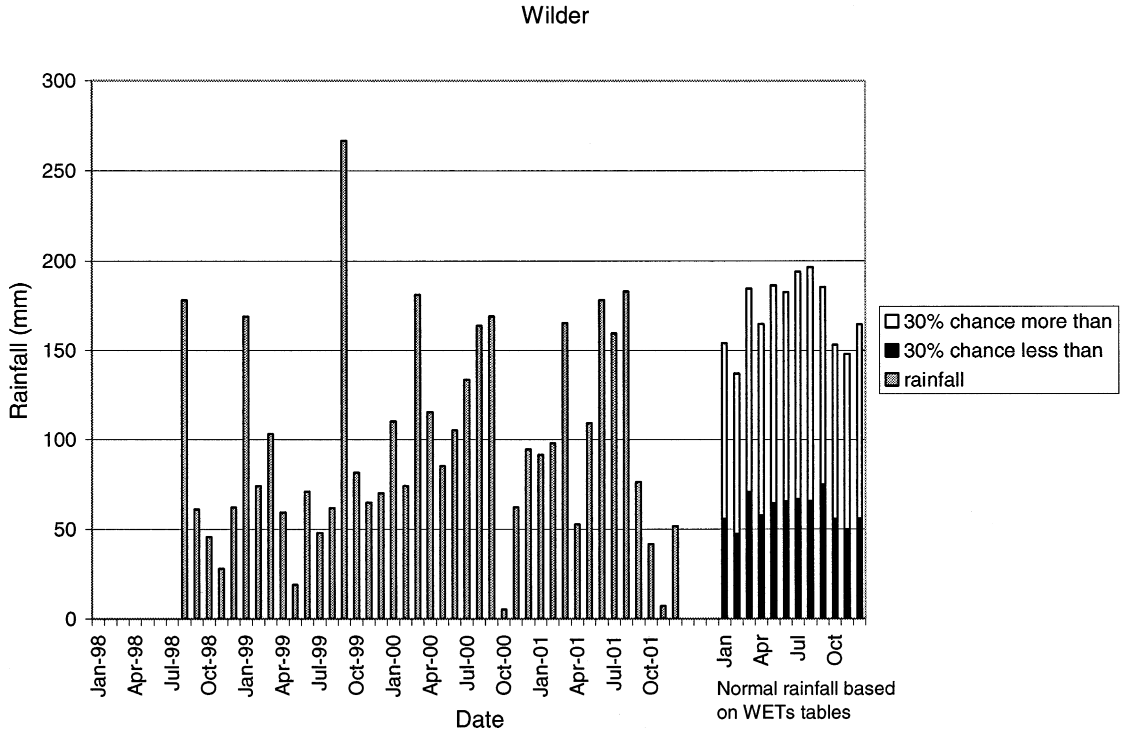

Figure 1. Continued.

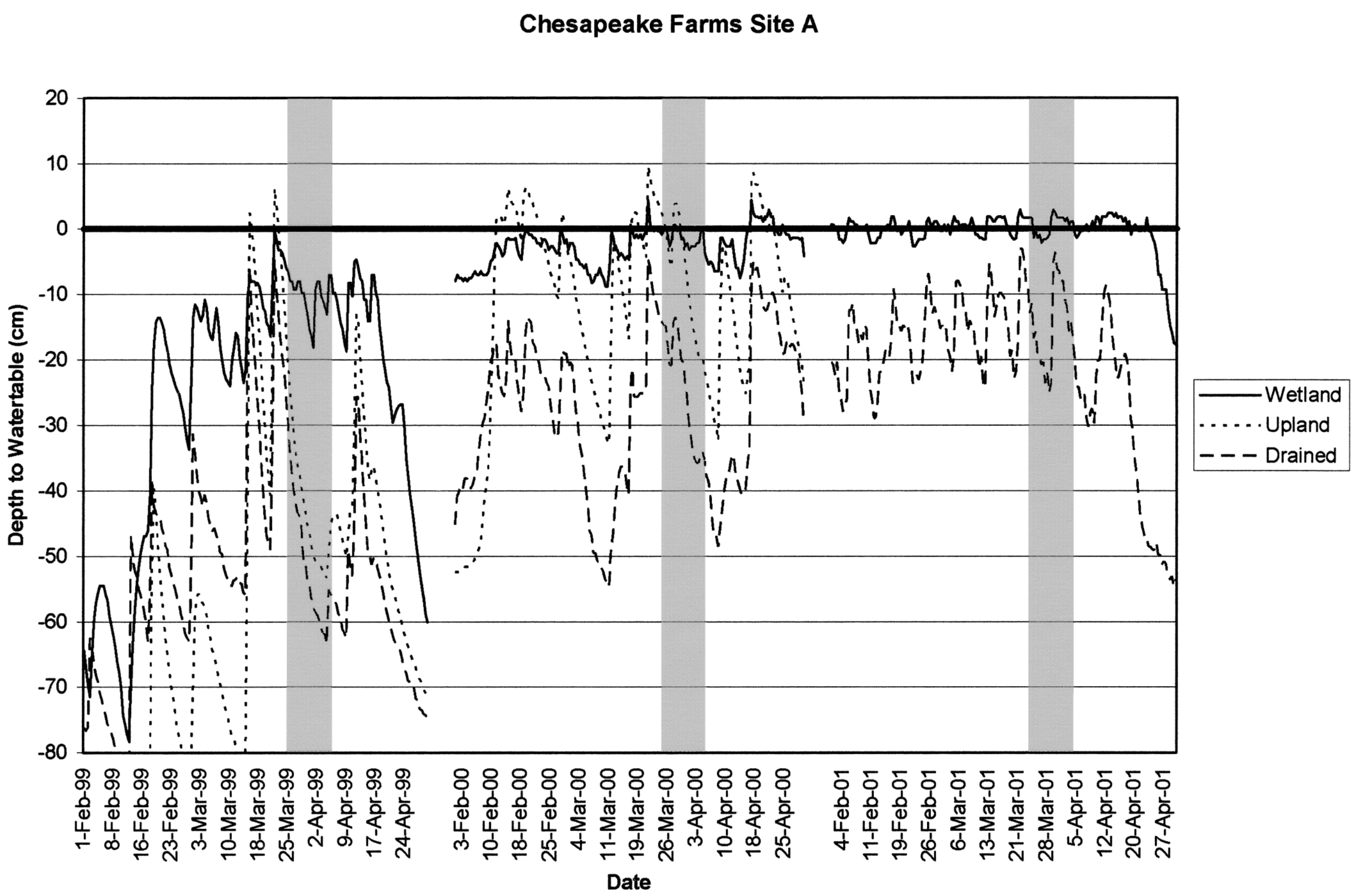

Figure 2. Hydroperiods for Chesapeake Farms A study site. Shaded areas represent the first 5% of the growing season.

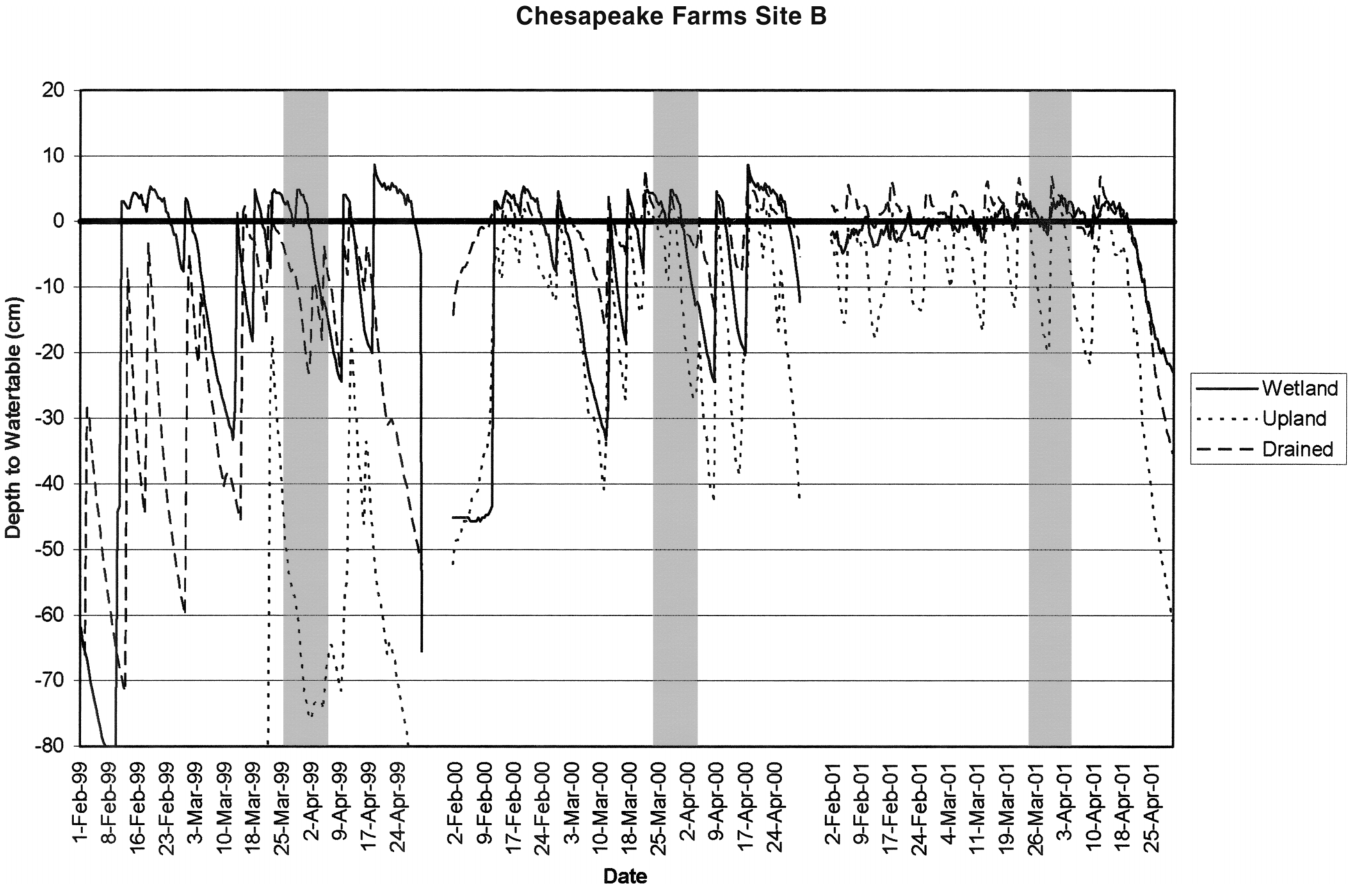

Figure 3. Hydroperiods for Chesapeake Farms B study site. Shaded areas represent the first 5% of the growing season.

tles could aid in jurisdictional determinations and on-site evaluations for mitigation purposes. A number of characteristics support their potential. Ectomycorrhizae development is strongly influenced by recent hydrology, but the effect of saturation on established ECM is not immediate, indicating that a reduction in oxygen is required (Stenstrom and Unestam 1987). Ectomycorrhizae form symbioses with a wide range of forest tree species. The symbiosis is easily detected with the naked eye by the presence of the mantle, which is stable and lasts for several months (Smith and Read 1997). To test the potential of ECM mantles as hydrology indicators, a three-year field project was conducted on the Delmarva Coastal Plain.

METHODS

Site Selection

Four naturally-forested sites representing soil catenas were chosen on the Delmarva Coastal Plain. Two sites were in Chesapeake Farms near Chestertown, Maryland, USA. The other sites were in Redden State Forest, near Redden, Delaware and Wilder Wildlife Management Area near Felton, Delaware. Plots were established in three sub-sites per site: a seasonally saturated wetland, a drained wetland, and an upland. Sub-sites were selected and characterized in March 1998. Uplands were characterized by the absence of any field indicator of hydric soils (USDA-NRCS 1998). Wetland sub-sites were characterized by the presence of a hydric soil indicator and soil saturation within 30 cm of the surface. Drained sub-sites were characterized by the presence of a hydric soil indicator and no soil saturation within 30 cm of the surface. All plots contained *Pinus taeda* L., which is especially dependent on ECM.

Soil Characterization

The soil in the center of each plot was characterized by a detailed description using USDA Natural Resources Conservation Service (NRCS) standards. An emphasis was placed on classifying the soils and identifying NRCS hydric soils indicators.

Site Instrumentation and Monitoring

Five wells were installed according to NRCS guidelines near the center of each plot. Two were automated

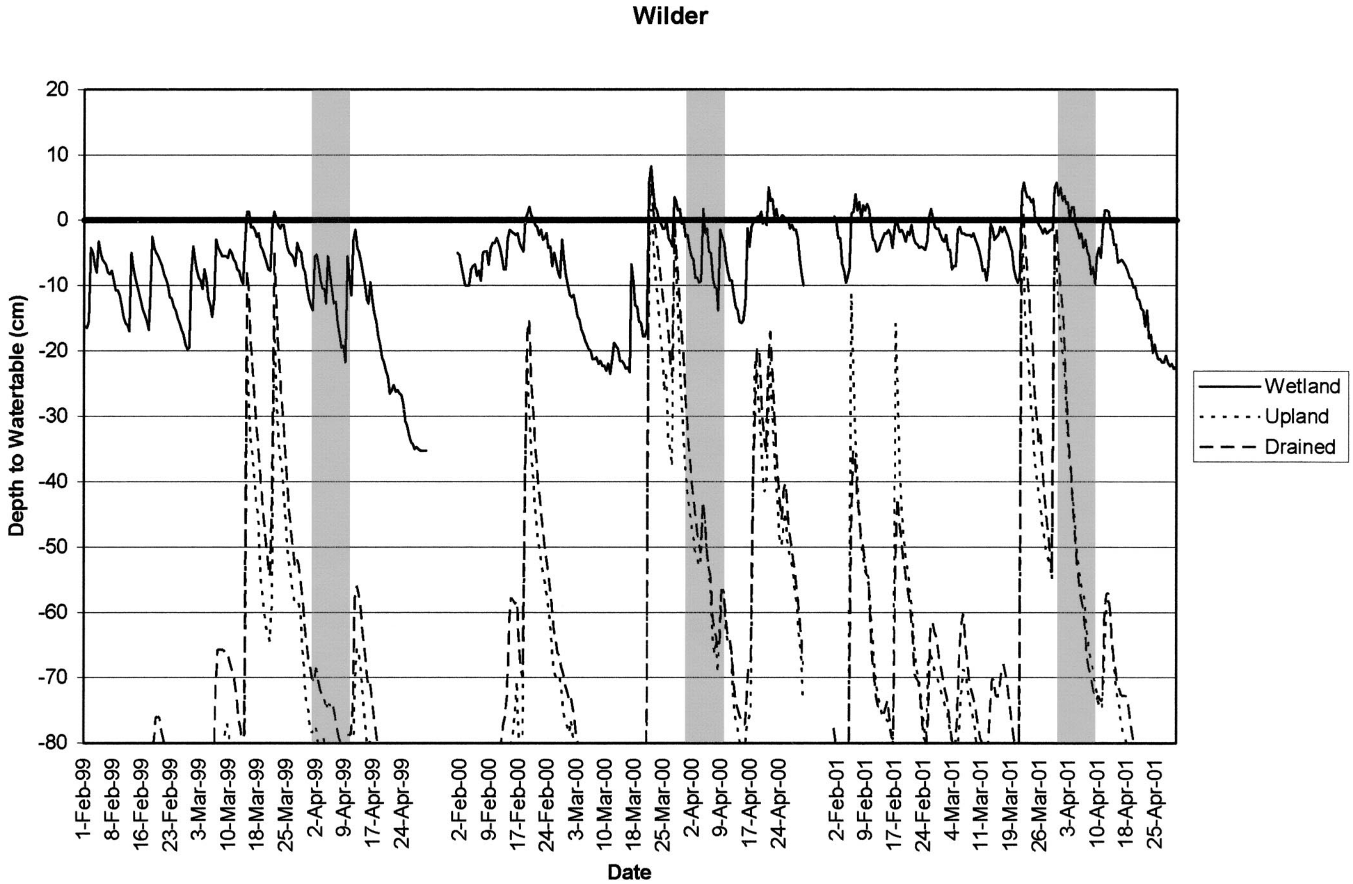

Figure 4. Hydroperiods for Wilder study site. Shaded areas represent the first 5% of the growing season.

recording wells (Remote Data Systems) installed to a depth of 1 m. They recorded the water-table depth twice per day. Three were simple wells (open slotted pipes, 7-cm diameter) installed to depths of 50 cm, 1 m, and 2 m. They were monitored with an electronic water-level indicator on a weekly basis except when precluded by severe weather or hunting season. Simple well data were used to verify automated well data and to provide information upon automated well failure. Platinum electrodes in conjunction with reference electrodes were used to make direct measurements of soil redox potentials. Five platinum electrodes were placed near the center of each plot and inserted into the soil to a depth of 15 cm at Redden and to a depth of 25 cm at the other sites. This discrepancy was due to textural differences that affect capillary rise. The electrodes were inserted in February each year and removed after the final yearly reading in April. Redox (voltage) measurements were taken after 5% and 12.5% of the growing season. The timing of these measurements was based on U.S. Army Corps of Engineers saturation/inundation criteria for jurisdictional wetlands (USACOE 1992). Growing season parameters were determined from USDA WETS tables representing the closest weather station. The growing season was considered to be that period when air temperatures exceed 28^{O} C for 50% of the years. Soil temperature and pH readings were taken along with the redox measurements to facilitate the conversion of voltage readings to redox potentials and to identify thresholds, respectively.

Plant Community Assessment

Plant communities were assessed with a plot/dominance procedure (10-m radius circular plot, 50/20 Rule, Basic Rule) in June and July of 1998 as outlined in the 1987 USACOE Wetland Delineation Manual (i.e., '1987 Manual') (Environmental Laboratory 1987).

ECM Sampling

Root sampling for ECM was conducted in March and August of 1999, 2000, and 2001. The presence of ECM was determined by the visual observation of mantles. Five *P. taeda* trees were randomly selected per plot. Two soil spade slices were taken per tree: at

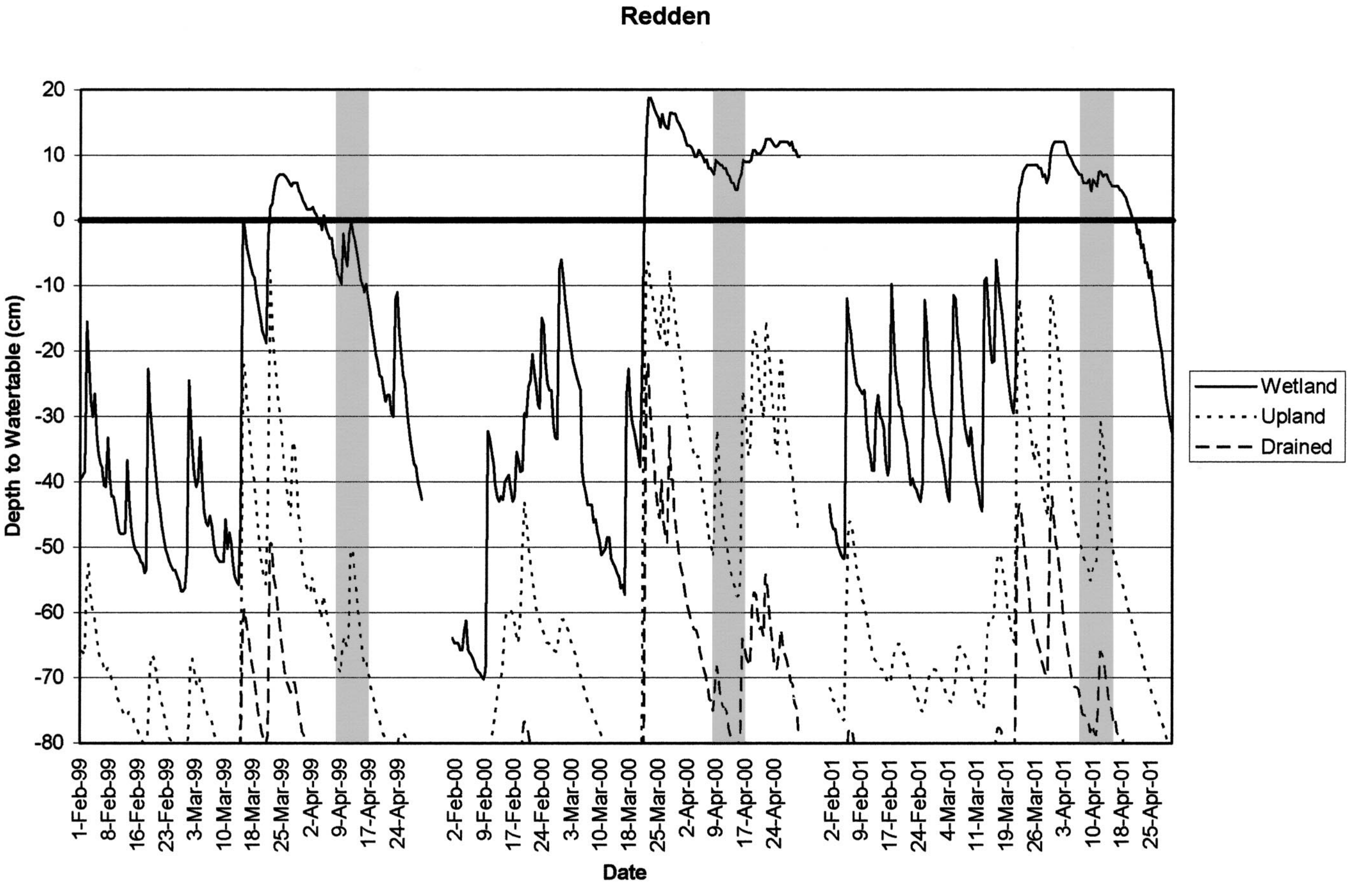

Figure 5. Hydroperiods for Redden study site. Shaded areas represent the first 5% of the growing season.

the drip line and midway between the drip line and the trunk. To preclude multiple samplings of a given soil location, each sampling date used a different compass point. Roots were collected and separated by depth: O horizons and 5 cm increments of the mineral soil to a depth of 20 cm.

Statistical Analyses

In total, this study has six factors. Year and location are environmental factors and are considered random. Treatment (upland, wetland, or drained wetland), method (sampling at the drip line or midway between the drip line and the trunk), season (March sampling versus August sampling), and depth are fixed factors. From the hypothesis that mantles are found at different depths for uplands and wetlands, we realized that it would be essential to allow heteroscedasticity for the depth of sample. Thus, separate variances were estimated for each sampling depth. The Mixed procedure of SAS- (Litell et al. 1996) was used to perform the computations. With the given data and stipulation of heteroscedasticity for depth, we found it infeasible to estimate interactions with year and location. Models with these terms would fail to converge or would be otherwise unusable. We chose to do separate analyses for each year and to include location as a random factor with no interactions. For the fixed factors all the main effects, two-way, three-way, and four-way interactions were included in the model. While no tests of interaction of the fixed factors with year are possible, comparing the separate analyses does allow us to assess possible interactions of the fixed factors with year.

RESULTS AND DISCUSSION

All wetland sites are palustrine and would fit the HGM (hydrogeomorphic) classification of mineral soil flats. Mineral soil flats are relatively level wetlands in which precipitation is the primary source of water, and water flow is primarily vertical and of low intensity. Drainage of the Wilder, Redden, and Chesapeake Farms B drained sub-sites was due to ditching. No ditches were evident for the Chesapeake Farms A sites; drainage was assumed to be due to tile drainage. The Chesapeake Farms B drained sub-site showed altered wetland hydrology starting in 1999. Inspection of the ditch revealed that a natural dam had formed,

Table 2. Soil Eh (mV) at 5% and 12.5% into the growing season and the Eh threshold for reducing conditions. Bold numbers are below the threshold.

	Upland				Wetland				Drained			
Date	Soil Eh			Threshold	Soil Eh			Threshold	Soil Eh			Threshold
Chesapeake Farms A												
4/7/99	333	562	564	317	**154**	**154**	**163**	319	383	371	390	336
4/21/99	352	546	569	314	**140**	**163**	**237**	323	353	365	355	333
4/10/00	**88**	369	426	332	**−53**	**−43**	**−42**	321	**−50**	**−19**	**33**	327
4/24/00	**57**	326	413	325	**−113**	**−96**	**−55**	315	**−46**	**−23**	**−19**	329
4/10/01	**10**	**19**	**48**	319	**−21**	**−15**	**34**	322	**8**	**12**	**14**	319
4/24/01	**0**	487	512	323	**−44**	**−43**	**30**	315	**−13**	**5**	**30**	327
Chesapeake Farms B												
4/7/99	**295**	580	627	327	**201**	552	557	310	373	396	479	334
4/21/99	**292**	568	615	322	**198**	539	542	313	**−163**	**164**	**261**	331
4/10/00	**21**	**148**	333	319	**−22**	**−5**	**199**	318	**−2**	**2**	**155**	334
4/24/00	**45**	**87**	330	323	**−8**	**9**	**126**	321	**−25**	**−17**	**133**	328
4/10/01	**9**	**49**	**62**	314	**−7**	**3**	**8**	314	**−12**	**97**	**163**	325
4/24/01	**12**	**250**	**283**	318	**−14**	**−9**	**−7**	317	**16**	**55**	**154**	333
Redden												
4/20/99	480	485	488	378	**−18**	**134**	**193**	382	**233**	489	637	380
5/4/99	**270**	472	534	380	**22**	**169**	**184**	384	**247**	601	634	377
4/21/00	**379**	485	537	381	**16**	**66**	**70**	381	616	634	643	383
5/7/00	**354**	472	539	369	**3**	**17**	**69**	388	585	596	602	379
4/17/01	**26**	543	611	393	**−68**	**−26**	**−13**	386	650	658	661	384
5/8/01	514	577	598	387	**28**	**190**	**215**	382	654	661	662	381
Wilder												
4/13/99	**78**	**270**	438	327	**−62**	**−57**	**268**	348	**297**	630	634	335
4/27/99	**183**	621	635	330	**123**	**147**	**271**	355	**312**	648	658	333
4/9/00	**−116**	**39**	599	334	**70**	**112**	**134**	363	511	526	615	354
4/30/00	**−13**	**80**	617	328	**37**	**66**	**112**	365	537	628	636	349
4/10/01	**351**	483	575	361	**40**	**86**	**175**	379	497	526	527	376
5/1/01	527	584	620	354	**44**	**158**	388	368	601	629	636	369

Table 3. Probability levels for fixed effects on number of roots with mantles. Bold numbers indicate significance at the 95% level of probability.

	Pr > F		
Effect	1999	2000	2001
Treatment (T)	0.200	0.069	**0.001**
Season (S)	**0.001**	0.364	0.994
T × S	0.491	0.931	0.704
Depth (D)	**<0.001**	**<0.001**	**<0.001**
T × D	**0.003**	**0.031**	**0.004**
S × D	**0.002**	0.458	0.350
T × S × D	0.750	0.914	0.450
Method (M)	0.805	0.630	0.422
T × M	0.470	0.854	0.971
S × M	0.931	0.273	**0.049**
T × S × M	0.323	0.386	0.662
M × D	0.454	0.896	0.901
T × M × D	0.423	0.734	0.905
S × M × D	0.439	0.820	0.518
T × S × M × D	0.120	0.385	0.416

which compromised drainage. From that point on, this site was considered to be a wetland.

Soil classifications and field indicators of hydric soils for the sub-sites are presented in Table 1. The hydric soil field indicators of S7, dark surface, and F3 depleted matrix (USDA-NRCS 1998) correspond to the 1987 Manual indicators of high organic matter content in a sandy soil and low chroma matrix, respectively. All wetland and drained soils would be categorized as poorly drained, while the upland soils were somewhat poor to moderately-well drained.

A hydrophytic plant community (Basic Rule-more than 50% of the dominant plant species have an indicator status of FAC or wetter) was present in all plots except the Wilder upland. *Liquidambar styraciflua* L., *Acer rubrum* L., and *Pinus taeda* were dominant in all wetlands, and this combination is representative of seasonally-saturated wetlands on the Delmarva Coastal Plain. Also common to the sub-sites and representative of seasonally-saturated wetlands and ad-

Table 4. Effect of depth on number of roots with mantles for wetlands, uplands, and drained wetlands at six sampling dates.

Site	O Horizon	Depth (cm) 0–5	5–10	10–15	15–20	O Horizon	Depth (cm) 0–5	5–10	10–15	15–20
	March 1999					August 1999				
Red. Wet.	13	12	2	0	0	10	1	0	0	0
Wil. Wet.	12	1	0	0	0	3	2	0	0	0
CFA Wet.	7	8	0	0	0	3	4	0	0	0
CFB Wet.	6	2	0	0	0	2	2	0	0	1
CFB Drn.	6	2	0	0	0	3	3	0	0	0
Red. Upl.	35	5	2	0	0	7	1	5	0	0
Wil. Upl.	8	5	5	5	2	3	6	2	4	0
CFA Upl.	0	7	6	4	0	0	2	1	0	0
CFB Upl.	3	11	3	3	0	5	1	2	3	0
Red. Drn.	48	8	6	0	0	10	0	4	1	0
Wil. Drn.	12	2	6	2	0	0	2	4	3	0
CFA Drn.	0	6	8	2	0	0	6	1	3	1
	March 2000					August 2000				
Red. Wet.	4	0	1	0	0	28	1	0	0	0
Wil. Wet.	8	0	0	0	0	6	2	1	0	1
CFA Wet.	7	4	0	0	0	9	1	0	0	0
CFB Wet.	10	4	0	0	0	5	1	0	0	0
CFB Drn.	4	4	0	0	0	3	1	0	0	0
Red. Upl.	4	2	7	1	0	48	6	5	0	0
Wil. Upl.	8	16	8	2	0	4	4	3	2	0
CFA Upl.	2	2	0	0	0	1	4	1	0	0
CFB Upl.	0	5	1	1	0	5	0	0	1	0
Red. Drn.	18	5	3	0	0	32	6	1	2	0
Wil. Drn.	14	9	4	5	2	9	5	7	5	1
CFA Drn.	8	6	1	0	0	6	8	1	0	0
	March 2001					August 2001				
Red. Wet.	9	2	0	0	0	15	4	0	0	0
Wil. Wet.	11	5	0	0	0	9	4	0	0	0
CFA Wet.	6	5	0	0	0	11	7	0	0	0
CFB Wet.	8	2	0	0	0	3	5	1	0	0
CFB Drn.	2	1	0	0	0	4	4	0	0	0
Red. Upl.	14	4	2	1	0	6	1	9	0	0
Wil. Upl.	7	11	4	5	0	9	5	8	4	2
CFA Upl.	4	1	0	0	0	1	3	1	0	0
CFB Upl.	5	2	1	0	0	5	3	1	0	0
Red. Drn.	41	1	3	2	0	10	3	13	5	0
Wil. Drn.	19	10	13	6	3	20	16	10	7	0
CFA Drn.	2	1	0	0	0	2	3	1	0	0

jacent uplands was *Nyssa sylvatica* L. *Pinus taeda* was dominant in all plots except the uplands at Wilder and Redden. However, there were enough individuals to allow for ECM sampling.

Hydrology indicators were drawn from the 1987 Manual. All wetlands were saturated to the soil surface in March and met the FAC-neutral test. In addition, the Chesapeake Farms A wetland and the Redden wetland had blackened leaves on the ground. All wetlands and drained sub-sites had soil series that met the secondary indicator of 'local soil survey data' (soil water features tables). Therefore, all wetland sub-sites and the Chesapeake Farms B drained sub-site met the soil, plant community, and hydrology indicators for jurisdictional wetlands as outlined in the 1987 Manual.

Monthly and long term precipitation data are presented in Figure 1. Data for the duration of this study were collected from on-site simple rain gauges. Long-term (30 years) data represent a range for normal precipitation as defined by the 30^{th} to 70^{th} percentiles. Long-term data were collected from USDA WETS tables for the closest weather station. Considerable yearly variability existed in precipitation patterns and precipitation totals at all sites.

Table 5. Effect of treatment and depth on number of roots with mantles. ‘‘Wetlands’’ include CFB drained sub-site. ‘‘Uplands’’ include all other drained sub-sites. Values represent the totals for a given sampling date, three-year totals, and the percentage of the grand total for a given depth.

	Wetlands					Uplands				
		Depth (cm)					Depth (cm)			
Sample Date	O Horizon	0–5	5–10	10–15	15–20	O Horizon	0–5	5–10	10–15	15–20
March 1999	44	25	2	0	0	106	44	36	16	2
August 1999	21	12	0	0	1	25	18	19	14	1
March 2000	33	12	1	0	0	54	45	24	9	2
August 2000	51	6	1	0	1	105	33	18	10	1
March 2001	36	15	0	0	0	92	30	23	14	3
August 2001	42	24	1	0	0	53	34	43	16	2
Total	230	94	5	0	2	435	204	163	79	11
% total	69.5	28.4	1.5	0	0.6	48.8	22.9	18.5	8.9	12

Automated well data are presented in Figures 2–5. Only data from early February to late April are presented as, for purposes of this discussion, the depth to the seasonally-high water table and water-table depths when mantles are forming early in the growing season are critical. Shaded areas represent the first 5% of the growing season (10, 11, and 12 days for Redden, Wilder, and Chesapeake Farms, respectively) and can be used to help identify those situations where the wetland criteria were met. The 1987 Manual presents the wetland hydrology criteria as continuous saturation to the soil surface for 5% of the growing season. On the basis of soil texture, saturation to or near the surface can be inferred from a water table within 15 cm for Redden and 25 cm for the other sites (USDA, SCS 1994). The 87 Manual wetland hydrology criteria were met in the following situations: all wetlands each year, Chesapeake Farms A drained sub-site in 2000 and 2001, Chesapeake Farms B sub-site each year, Redden upland in 1999, and Chesapeake Farm uplands in 2000 and 2001. Both automated wells in the Chesapeake Farms A upland malfunctioned in 2001, and there is a gap in the data from early February to early May. Therefore, the hydrology interpretation is based on simple well data (not presented).

Table 6. Probability of finding a root with a mantle below a depth of 5 cm as affected by sample number for uplands and wetlands.

No.	Probability %		No.	Probability %	
Samples	Upland	Wetland	Samples	Upland	Wetland
1	25.0	2.0	11	95.8	19.9
2	43.8	4.0	12	96.8	21.5
3	57.8	5.9	13	97.6	23.1
4	68.4	7.8	14	98.2	24.6
5	76.3	9.6	15	98.7	26.1
6	82.2	11.4	16	99.0	27.6
7	86.7	13.2	17	99.2	29.1
8	90.9	14.9	18	99.4	30.5
9	92.5	16.6	19	99.6	31.9
10	94.4	18.3	20	99.7	33.2

The Chesapeake Farms soils are finer textured (silt loams) than those at Wilder (sandy loams) and Redden (loamy sands). We believe that these silt loam soils act as ‘sponges’ in that the upper part of the soil will become saturated after rain events even when the water table is relatively deep. However, these periods of saturation may be quite transient. Also, the well-developed argillic horizons in these soils often act as confining layers, which results in perching when the water table is dropping and artesian systems below the argillic horizon when the water table is rising. According to the 50 cm simple wells (data not presented), the water table in the Chesapeake Farms B upland never came to the surface, so the automated 1-m well data may be indicating a partial artesian effect.

Soil redox potential data are presented in Table 2. The sampling dates approximate 5% and 12.5% into the growing season. The three values for each date/location combination represent the lowest of five readings taken. For a soil to be considered to be anaerobic, the lowest three readings must be below the threshold value which varies with soil pH as follows: threshold Eh (mV)=595-(60xpH) (NTCHS, 2003). Also, the soil must be saturated at the depth of the electrodes. Saturation depth was inferred from water table-depths in the 50 cm simple wells and the expected capillary rise of 15 cm at Redden and 25 cm at the other sites. With the exception of the Redden wetland (second samplings of 1999 and 2001, only), the Eh criteria for anaerobiosis always agreed with the saturation criterion. It is possible that, because of the high organic matter content of that soil, capillary rise is greater than would be expected for a loamy sand soil. The important information to obtain from these data is that saturation led to the development of anaerobiosis (as opposed to oxyaquic conditions) and, in general, the lag period between saturation and anaerobiosis was approximately two weeks.

Sites chosen for this study would be a challenge to delineate as topographic breaks were minor, plant communities were dominated by FAC species, and the uplands had somewhat-poorly drained, but not hydric, soils. Such uplands will meet the wetland hydrology criteria in some years but not at sufficient frequency or duration to develop hydric soils. Therefore, they will not meet the jurisdictional criteria for wetlands. In this study, upland and drained sub-sites met the wetland hydrology criteria in some years. Therefore, we need to evaluate the mantle data from two different perspectives. For statistical purposes and to identify relationships, the 'uplands' and drained sub-sites (save Chesapeake Farms B) will be considered to be 'uplands.' However, since the wetland hydrology criteria were met in some years in upland and drained sub-sites, we also need to look at the relationship between mantle depth and hydrology for each individual site/year data set. This second approach will allow us to identify limits to the potential use of mantles as a hydrology indicator.

An analysis of variance for the mantle data is presented in Table 3. The wetlands include the Chesapeake Farms B drained sub-site because it displayed wetland hydrology during the course of this study. From a conceptual standpoint, the 'treatment/depth interaction' is the most important statistic because it verifies the initial hypothesis that the vertical distribution of mantles should be different in wetlands than in uplands. From a practical standpoint, the 'season' and 'season/depth interaction' effects in mantle distribution with depth in 1999 could have potential implications in the development of a protocol, as they could impact the temporal window when samples can be taken. We believe that the 'season' effects in 1999 were due to drought (see below) and that, in most years, a late summer sampling for mantles will produce a representative depth to the seasonally-high water table.

Mantle distribution with depth for individual samplings is presented in Table 4. As indicated by the statistical analysis, some factors such as 'depth' and the 'treatment x depth' interaction were consistently significant. The number of mantles consistently decreased with depths greater than 5 cm; this effect was greater in wetlands than in uplands and greater in uplands than in drained (except Chesapeake Farms B) sub-sites. These responses are clearly associated with differences in the seasonally-high water table. Two factors, 'season' and 'season x depth,' were significant only in 1999, the driest year. More mantles were found in August 2000 and August 2001 than in August 1999. This can be attributed to the summer drought of 1999. Ectomycorrhizae formation is reduced in both saturated soil and very dry soil (Fogel 1980, Lodge 1989). Large variability can be found in mantle distribution from sampling to sampling. Again, this is clearly associated with hydrologic factors. The drained sub-sites at Redden and Wilder were the driest and usually had the greatest numbers of mantles below the 5-cm depth. The Chesapeake Farms A upland had wetland hydrology in 2000 and 2001, but not in 1999. Its distribution of mantles was similar to the adjacent wetland in 2000 and 2001, but not in 1999. To address sampling variability further, there were thirty-three possible interactions over the three years of sampling. Only four were significant, three of which were the treatment x depth' interaction. Therefore, the sampling procedures and statistical analyses compensated for biological variability and identified useful treatment responses.

A summary of the mantle data is presented in Table 5. Each value represents the sum for all wetlands or all uplands for a given sampling date. The wetlands include the Chesapeake Farms B drained sub-site, as it displayed wetland hydrology during the course of this study. It is apparent that 5 cm represents a threshold depth, below which the presence of mantles may indicate a lack of wetland hydrology. For wetlands, of 331 roots with mantles, seven (2%) were found below the threshold depth. For uplands, 253 of 892 roots with mantles (28%) were found below the threshold depth. Therefore, under similar vegetative (the presence of plant species that form ECM) and soil (no excessive phosphorus levels) circumstances, we can expect to find more mantles below a 5-cm depth in uplands than in wetlands. The observation of one mantle below 5 cm, however, should not be interpreted as evidence that the site is not a wetland. Yearly variability in precipitation, micro-topography, and the presence of aerobic zones in saturated soil can all promote deeper formation of mantles in wetlands. However, the probability of finding a mantle below this threshold depth is much greater in uplands than in adjacent wetlands. Also, mantle depth was often restricted in these uplands when the wetland hydrology criteria were met. A more conservative implication would be that the observation of mantles below 5 cm is an indicator that the wetland hydrology criteria were not met that spring.

From a practical standpoint, it is important to have an understanding of the number of samples (spade slices) that need to be taken to distinguish uplands from wetlands on the basis of ECM mantles. Table 6 gives the probabilities of finding a mantle below 5 cm in a wetland or upland dependent on sample number. These probabilities are based on this data set and may be different for a different tree species or a different physiographic region. Of course, the probability of finding a mantle increases as sample size increases. For example, the chances of finding a mantle below 5 cm in a wetland

and an upland from one sample are 2% and 25%, respectively. For two samples, these probabilities increase to 43.8% and 4.0%, respectively. For twenty samples, the probabilities of finding the mantle below the threshold depth in an upland or wetland rises to 99.7% and 33.2%, respectively. The appropriate sample size (number of spade slices) should balance the intention to maximize the probability of finding a mantle in uplands below the threshold depth and minimize the probability of finding a mantle in wetlands below the threshold depth. It should also be practical. Our consensus is that six spade slices are appropriate. For six samples, the probabilities of finding a mantle below the threshold depth for uplands are 82% and 11% for wetlands.

Pinus taeda was targeted because it readily forms ectomycorrhizae, it is prevalent across a wide section of the upland-wetland continuum, it is a dominate species on the Delmarva Coastal, and it is easily identified. However, as indicated previously, ECM form on a significant number of tree families. We believe that the hydrology-ECM depth relationship found in this study should be similar for many other tree species. Also, although *P. taeda* was targeted, it was impossible to determine if the sampled roots were of that species. It certainly is plausible that other species were sampled.

This project clearly showed the relationship between the depth of ECM mantles and the presence or absence of wetland hydrology in an area. This information will be especially useful in areas that were once wetlands and have drainage ditches present, yet still exhibit indicators of hydric soils. The mantles may help distinguish areas that are effectively drained by ditches from areas in which the apparent water table has been lowered by ditches, but wetland hydrology is maintained by episaturation, as is common on the Delmarva Coastal Plain. The mantles may also be useful in identifying potential wetland restoration sites by identifying drained hydric soils, which obviously had wetland hydrology at one time and should be able to support wetland hydrology again, simply by compromising the mechanism for drainage. These results will supply a basis for future projects to address the potential for using the abundance and location of ECM mantles as an indicator of changes in hydrology of an area.

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